DOI: 10.7860/JCDR/2024/68621.19292

Original Article

Microbiology Section

Clinico-bacteriological Study and Molecular Detection of *Campylobacter* in Childhood Diarrhoea: A Cross-sectional Study

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ABSTRACT

Introduction: *Campylobacter* infections cause diarrhoeal diseases as frequently as Salmonella and Shigella infections. The prevalence of *Campylobacter* infection among children with acute diarrhoea in developing countries ranges from 5-35%. Diagnosing *Campylobacter* infections is challenging as the organism is difficult to isolate, grow, and identify. Currently, no best-practice clinical or public health laboratory guidelines exist for laboratory diagnosis of *Campylobacter* infections.

Aim: To explore the clinical and bacterial aspects of childhood diarrhoea, emphasising the prevalence and molecular detection of *Campylobacter*.

Materials and Methods: A hospital-based cross-sectional descriptive study was conducted with 55 stool samples of children under five with diarrhoea or dysentery at the Department of Microbiology, JSS Medical College, Mysuru, Karnataka, India, from October 2016 to September 2017. All stool samples were inoculated onto *Campylobacter* selective and non selective media with filtration and incubated in microaerophilic conditions. The culture isolates were identified by standard phenotypic tests. Molecular characterisation of *Campylobacter* was performed targeting the *Campylobacter* adhesion to fibronectin F (cadF) gene. The presence of a phylogenetically conserved 16S ribosomal Ribonucleic Acid (16SrRNA) domain was studied, followed by specific detection of pathogenic *Campylobacter* species. The Statistical Package for Social Sciences (SPSS) version 22.0 was used for statistical analysis. Descriptive statistics like percentage, mean, and Standard Deviation (SD) were applied.

Results: *Campylobacter* was isolated by culture in one out of 55 stool samples. The isolate was confirmed to be *Campylobacter jejuni* by phenotypic tests. *Campylobacter* genus-level Polymerase Chain Reaction (PCR) was positive for 6 samples (10.9%). Six positive samples were subjected to species-level PCR; all were positive for *C. jejuni.* Out of 55 stool samples, two diarrheagenic *Escherichia coli*, two *Shigella sonnei*, one *Shigella dysenteriae*, and one *Salmonella enterica serovar Typhi* were also identified.

Conclusion: Culture is insufficiently sensitive for diagnosing *Campylobacter* infection compared with nucleic acid-based diagnostics. Nucleic acid-based diagnostics offer increased sensitivity, can determine both the presence and burden of infection, and can distinguish between *Campylobacter* infections at the species level. Therefore, PCR is recommended, if feasible, as the preferred diagnostic modality for detecting *Campylobacter* infection.

Keywords: *Campylobacter* infection, Dysentery, Molecular diagnostic techniques

INTRODUCTION

Diarrhoea is one of the most common diseases causing significant morbidity and mortality in children in developing countries. Diarrhoea is defined as the passage of abnormally liquid or unformed stools at an increased frequency. It is classified as acute if the duration is less than two weeks, persistent if lasting 2-4 weeks, and chronic if lasting more than four weeks [1]. Diarrhoea significantly contributes to the high prevalence of malnutrition in young children, predisposing them to a vicious cycle of diarrhoea and malnutrition, making it a major public health problem [2].

Campylobacteriosis is a zoonotic disease observed in most parts of the world. It is rapidly becoming the most commonly recognised cause of bacterial gastroenteritis in humans, estimated to cause 5-14% of diarrhoea cases worldwide. *Campylobacter* infections are found to cause diarrhoeal disease 2-7 times more frequently than infections with Salmonella species and Shigella species, or are usually associated with *Escherichia coli* O157:H7 [3]. The high incidence of *Campylobacter* diarrhoea, its duration, and possible sequelae make it highly important from a socio-economic perspective [4]. In developing countries, *Campylobacter jejuni* is identified as one of the top five causes of diarrhoea among children aged 24-59 months, contributing significantly to the burden of diarrhoea. In India, *C. jejuni* was the third most common cause of diarrhoea in children aged 2-5 years, following rotavirus and Shigella [5]. In South India, the prevalence of *Campylobacter* infections among children with diarrhoea was 4.5% [6].

Campylobacter is a Gram-negative curved, fastidious organism that requires a microaerophilic environment for growth. The optimum temperature for its growth is 30-37°C. The two species most commonly associated with human disease are *C. jejuni* and *C. coli. Campylobacter jejuni* accounts for over 80% of *Campylobacter*-related human illnesses, while *C. coli* accounts for 18.6% of human cases [7].

Campylobacter infection primarily occurs in infants, older people, and patients with underlying diseases [4]. Symptoms include diarrhoea (sometimes bloody), nausea, abdominal pain, fever, headache, and vomiting. The incubation period is usually 2-5 days, with illness generally lasting 2-10 days. A unique feature of the disease is the severity of abdominal pain, which may become continuous and intense enough to mimic acute appendicitis [8]. *Campylobacter* has four main virulence attributes: motility, adherence, invasion, and toxin production. The combination of its spiral shape and flagella allows for rapid motility, enabling the organisms to penetrate the intestinal lining, unlike other intestinal bacteria [9].

Campylobacter is diagnosed through direct microscopic examination to detect darting or tumbling motility of spiral rods by culturing faeces or rectal swabs on selective media. Enzyme immunoassay can also be used for diagnosis [10]. Polymerase Chain Reaction (PCR) has high sensitivity in detecting *Campylobacter* and can distinguish between *Campylobacter* infections at the species level. Diagnosis can be challenging because the organism is difficult to isolate, grow, and identify. Direct plating onto *Campylobacter* selective medium, followed

by incubation at 42°C under microaerobic conditions for 72 hours, has long been considered the gold standard for diagnosis [11].

Complications of *Campylobacter* infections occur due to direct spread from the gastrointestinal tract and can include cholecystitis, pancreatitis, peritonitis, and massive gastrointestinal haemorrhage [3]. Bacteraemia can arise due to inadequate therapy; sometimes, sequelae can be seen in the form of Guillain-Barre syndrome and its variant, Miller-Fischer syndrome [4]. Correction of electrolyte abnormalities and rehydration are the mainstays of treatment for *Campylobacter enteritis*. Antimicrobial therapy is recommended in invasive cases or to eliminate the carrier state [12]. Erythromycin and Ciprofloxacin are the drugs of choice, but resistance to these drugs is increasing, especially in developing countries [13]. It is necessary to detect *Campylobacter* from diarrhoeic stool to initiate prompt and appropriate antimicrobial therapy, which can reduce infection duration, severity, and complications [14].

Applying traditional laboratory methods and molecular techniques in identifying *Campylobacter* infections facilitates understanding the burden among under-five children, helping prevent unforeseen complications and mitigating antibiotic resistance by guiding judicious antibiotic use. Hence, the present study aimed to explore the clinical and bacterial aspects of childhood diarrhoea, emphasising the prevalence and molecular detection of *Campylobacter*. The primary objective is to estimate the prevalence of *Campylobacter* infections and investigate the clinical manifestations in under-five children. Secondary objectives are to compare *Campylobacter* culture and PCR results and to find out the association of different variables with *Campylobacter* gastroenteritis.

MATERIALS AND METHODS

A hospital-based cross-sectional descriptive study was conducted at Department of Microbiology, JSS Medical College, Mysuru, Karnataka, India, for one year (October 2016-September 2017). A structured questionnaire, which included the demographic data of the patient and the clinical history, was used. The study was approved by the JSS Medical College and Hospital Institutional Ethics Committee (letter No. JSSMC/PG/658/2015-16). A purposive sampling method was used, and informed consent was obtained from the parents/guardians accompanying the children before collecting their stool samples for the study. All procedures followed were in accordance with the ethical standards of the institutional committee on human experimentation and the Helsinki Declaration.

Inclusion and Exclusion criteria: Under-five children with acute diarrhoea or dysentery presented to the paediatrics department during the study period were included. Malnourished, immunocompromised children, and children on macrolides, quinolone, and prolonged steroids were excluded.

Sample size calculation: The required sample size of 48 was calculated using the formula [15] $Z^2*p*(1-p)/d^2$, where 'Z' represents the standard normal variable (1.96), p is a proportion based on a previous study conducted in Karnataka [16] (0.32), d is the absolute precision (5%), and a confidence level of 95%. Factoring in a non response rate of 7 (15%), the total sample size was determined to be 55.

Study Procedure

Sample collection and storage: Stool samples were collected in dry, sterile, leak-proof, wide-mouth containers. Most samples were processed immediately, while a few were stored at 2°C to 8°C and processed within 72 hours for *Campylobacter* culture. For PCR, stool samples were suspended in 1X Phosphate Buffered Saline (1X-PBS). Approximately 200 to 300 mg of stool sample was resuspended in 1 mL of 1X-PBS, thoroughly mixed to create a uniform stool suspension, and stored at -80°C in an ultra-low-temperature freezer (New Brunswick Scientific) until further processing.

Microscopy: Saline and iodine mounts were performed for faecal leukocytes, Red Blood Cells (RBCs), and motile bacteria to rule out parasitic causes. Gram's staining was conducted on suspected samples (with characteristic motility or presence of thin curved/spiral forms) for the microscopic morphology of *Campylobacter*. Modified Gram's stain with safranin counterstaining for ten minutes was utilised. Standard laboratory methods were adopted whenever other enteric pathogens were suspected on microscopy to isolate/identify them. Samples were inoculated onto one selective and one non selective medium. Two media were used simultaneously: the blood-free *Campylobacter* selective medium with supplement (Himedia, Mumbai, India) and 5% sheep blood agar (Himedia). Samples were inoculated after filtration through a 0.45 µm membrane filter (Himedia, Mumbai, India). The inoculated media were then incubated in a microaerophilic atmosphere using candle jars (Oxoid) at 37°C. In each 2.5 L capacity jar, seven plates of 90 mm diameter could be kept simultaneously, with five to six wax candles lit to create the microaerophilic atmosphere. A filtration method was also employed with non selective and selective media to enhance the recovery of *Campylobacter*. This was achieved by placing a filter (0.45 micrometers pore size, Himedia) on the agar surface and adding 2-3 drops of stool onto it. The plate was then incubated upright. After 60 minutes at 37°C, the filter was removed, and the plates were re-incubated in a microaerophilic atmosphere. As the organisms were motile and capable of migrating through the filter, they formed isolated colonies on the culture plates. Suspected colonies from the plates were checked by Gram's stain (slender, curved, "gull-wing" shaped Gram-negative rod); a wet preparation of the organism was made for characteristic darting motility, oxidase, and catalase tests. *Pseudomonas aeruginosa* ATCC 9027 was used as a positive control for the oxidase test, and *Staphylococcus aureus* ATCC 25293 was used for the catalase test.

The same stool samples were simultaneously screened for other pathogens such as *Salmonella*, *Shigella*, *Vibrio*, *Aeromonas*, *Diarrhoeagenic Escherichia coli*, and intestinal parasites. For screening these pathogens, all stool samples were plated on selective media such as MacConkey's agar, Xylose lactose deoxycholate, and Thiosulfate citrate bile salt agar. Identification was conducted using the Vitek system.

Molecular detection by Polymerase Chain Reaction (PCR): Briefly, 1 mL of uniformly mixed stool suspension was centrifuged at 600 Rotations Per Minute (rpm) for five minutes to pellet down coarse undigested materials. This was followed by the separation of the supernatant in a fresh sterile Microcentrifuge Tube (MCT), which was further centrifuged at 12,000 rpm for three minutes to sediment complete cellular fractions, including microbial cells. The cellular-rich pellet was washed with 1 mL of cold acetone (#MB179-500 mL, Himedia) to remove several dissolved inhibiting substances in the stool. It was then washed twice with 1 mL of 1X-Dulbecco's Phosphate Buffered Saline (#TS1006-1L, Himedia) to remove excess acetone, including several chromogenic substances. The washed pellet was subjected to Deoxyribonucleic Acid (DNA) extraction using the HiPurA™ Stool DNA Purification kit (#MB544, Himedia), following the manufacturer's protocol with slight modifications. The kit also contained a unique proprietary solution called Inhibitor Removal Solution (IRSH), which removes PCR inhibitory substances by precipitation during the early steps of extraction. The eluted DNA was further subjected to quantification and purity assessment using Nanodrop (DeNovix), followed by agarose gel electrophoresis to ensure the presence of good quality DNA for downstream applications.

The molecular analysis was conducted in three stages. The first stage of the molecular study involved the broad-range detection of the 16S ribosomal Deoxyribonucleic Acid (rDNA) gene as an internal control to validate the presence of pathogenic bacterial DNA in the sample extract. Negative results were excluded from the study as these samples did not present with bacterial DNA. Both positive and

negative controls were included in the reaction. Pure clinical isolates of *Campylobacter* species (*C. jejuni*) were used as a positive control. Primers (U1/U2) for detecting a pathogenic group of bacteria were adopted from Lu JJ et al., and procured from Eurofins genomics. The primer sequences included U1: 5'-CCAGCAGCCGCGGTAATACG-3' and U2: 5'-ATCGG(C/T)TACCTTGTTACGACTTC-3' [17].

The 2nd stage of the molecular study involved the universal detection of *Campylobacter* species using a universal primer mix, and the 3rd stage included the characterisation of *Campylobacter* universal positive samples into two pathogenic species associated with gastrointestinal pathologies, including *C. jejuni* and *C. coli.* Both universal and species-specific primer mixes were procured from Helini Biomolecules along with their respective positive controls. A putative virulence determinant, cadF (*Campylobacter* adhesion to fibronectin F) gene, was targeted for designing primers for *C. jejuni* and *C. coli.* This gene encodes an adhesion and fibronectin-binding protein involved in the invasion process, thereby influencing microfilament organisation in host cells [18].

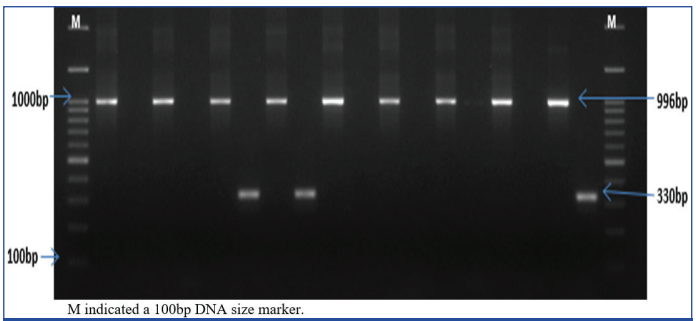
The PCR reaction was carried out in a 30 µL volume, including 1X PCR buffer (#M0273S, NEB) containing 10 mM Tris-HCl (pH 8.8 at 25°C), 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, dNTPs mix (0.5 mM), MgCl2 (0.5 mM), BSA 0.8 mg/mL (#SH30574.20, HyClone), Taq DNA polymerase 1U (#M0273S, NEB). The primer concentrations included 0.01 µM (16S rDNA) and 0.3 µM (*Campylobacter*) in each PCR reaction. Finally, 3 µL of a sample or respective control was added. Gradient PCR was performed (Mastercycler gradient, Eppendorf) to standardise PCR conditions, especially primer annealing. The common PCR conditions for all target genes amplification included initial denaturation at 95°C for five minutes, followed by 35 cycles of denaturation (95°C, 45 seconds), annealing (58°C, 45 seconds), and extension (72°C, 45 seconds), which was followed by a final extension at 72°C for five minutes. Post-PCR products were resolved on a 2.5% agarose gel with ethidium bromide (10 mg/mL) using electrophoresis at 50V. Finally, DNA bands were visualised using the gel documentation unit (exposure time eight seconds in TLUM mid-wave photo mode). Results were analysed based on the presence or absence of specific amplicons with product sizes of 996 bp (16S rDNA), 330 bp (*Campylobacter* universal), 335 bp (*Campylobacter jejuni*), and 365 bp (*Campylobacter coli*). A 100 bp DNA size marker was included to estimate the size of the PCR products. Confirmation of PCR products by Sanger's sequencing: PCR products from representative samples were subjected to column purification using the QIA quick PCR Purification Kit (#28104, Qiagen). Further, purified PCR products were subjected to cycle sequencing using dye terminator chemistry, i.e., BigDye Terminator v3.1 and Polymer POP7. Forward and reverse reactions were carried out in separate reactions using respective primers. Sanger's sequencing (ABI3730XL-96, Applied-Biosystems) was employed, and the respective chromatogram was developed. Finally, nucleotide sequences were aligned with the respective bacterial genome database available in the NCBI gene bank, using multiple sequence alignment tools, i.e., Clustal Omega software. All products for both forward and reverse sequenced reactions found 99-100% homologies with the expected targets, i.e., *Campylobacter* species, with an E (Expected) value near to zero, which confirmed the source of the DNA sample as *Campylobacter* in the given stool sample.

STATISTICAL ANALYSIS

The data entry and statistical analysis were done using Microsoft excel and SPSS version 22.0. Descriptive statistics like percentage, mean, and standard deviation were applied. The Chi-square test was applied to find the association between variables and *Campylobacter* gastroenteritis. Fisher's exact test was applied where 20% of expected values were less than 5. A significant association was considered at $p<0.05$.

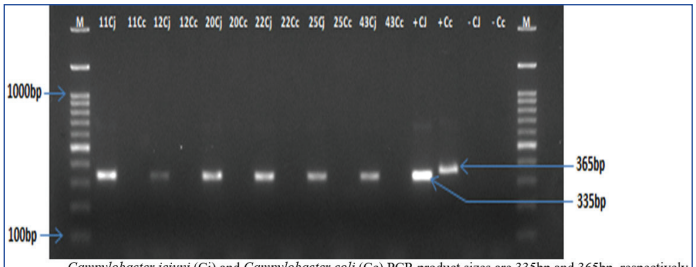
RESULTS

Among the 55 stool samples of under-five children who presented with complaints of diarrhoea, dysentery, and acute gastroenteritis were included. Among the study participants, 23 (41.82%) were infants, 13 (23.64%) belonged to the age group of 12-24 months, and the remaining 19 (34.54%) belonged to 24-60 months. Out of the 55 under-five children, 35 (63.6%) were males and 20 (36.4%) were females. Of the 55 children, 12 presented with only diarrhoea, 26 with diarrhoea and fever, and 17 with diarrhoea and other symptoms such as vomiting, abdominal pain, and febrile seizures. Among the study participants, 17 (30.9%) were clinically diagnosed with acute gastroenteritis with no dehydration, 12 (21.81%) with acute gastroenteritis with some dehydration, 9 (16.36%) with pyrexia of unknown origin, 8 (14.55%) with dengue fever, 5 (9.09%) with respiratory infections, 2 (3.64%) with bacillary dysentery, and 2 (3.64%) with viral fevers. Among the 55 study subjects, 39 (70.9%) were on antibiotics such as Cephalosporins and Betalactam-beta-lactamase inhibitor combinations, while 16 (29.09%) were not on antibiotics at the time of sample collection. Out of the 55 patients, 2 (3.64%) had a history of animal exposure, while the rest had no history of animal exposure. Stool macroscopy revealed the presence of mucus in 28 (50.9%) and blood in 2 (3.6%) stool samples. Stool consistency was liquid in 34 (61.8%) and semisolid in 21 (38.2%) samples. On stool microscopy, 16 (29.1%) samples were normal, 30 (54.5%) had inflammatory cells, and 9 (16.4%) had both red blood cells and inflammatory cells. Out of the 55 stool samples, six other intestinal pathogens and one *Campylobacter* were isolated by culture. Among the six pathogens, two were Diarrheagenic *Escherichia coli*, two were *Shigella sonnei*, one was *Shigella dysenteriae*, and one was *Salmonella Typhi*. Among the 55 stool samples, *Campylobacter* culture was positive in only one sample. Suspected tiny grey-coloured colonies on a selective medium were phenotypically identified as *Campylobacter* by Gram stain morphology, characteristic motility, oxidase, and catalase tests. Genomic DNA isolation using a spin column-based method yielded intact DNA bands from all 55 stool samples when observed after electrophoresis. Further DNA purity assessment was found to be within the acceptable limit (260/280 ratio: 1.6-1.9) when analysed using nanodrop. The PCR result revealed successful amplification of 16S rDNA from all 55 samples with an expected band size of 996 bp. The presence of the 16S rDNA band validated the source of DNA as a bacterial population. Further, these bacterial positive DNA samples were studied for the presence of *Campylobacter*-specific genes. Six (10.9%) samples were identified as positive cases for *Campylobacter* out of the 55 clinically suspected samples [Table/Fig-1].



[Table/Fig-1]: Molecular identification of *Campylobacter* universal with broad range 16S rDNA PCR.

Campylobacter-positive samples were finally subjected to species identification. All six *Campylobacter*-positive samples showed the presence of *C. jejuni*, and none showed the presence of *C. coli*, neither as a pure isolate nor as a mixed isolate with *C. jejuni*, indicating that *C. jejuni* is solely responsible for the enteric pathology associated with *Campylobacteriosis* among the study population [Table/Fig-2]. In the present study, no statistically significant association was found between various factors (gender, age, exposure to antibiotics, exposure to animals) and PCR results. However, the prevalence of *Campylobacter* infection was higher in children clinically diagnosed with acute gastroenteritis with some dehydration (4, 33.3%) compared to other clinical diagnosis, and this difference was found to be statistically significant (p-value=0.036) as shown in [Table/Fig-3].



Campylobacter jejuni (Cj) and *Campylobacter coli* (Cc) PCR product sizes are 335bp and 365bp, respectively. M indicated a 100bp DNA marker, and +Cj and +Cc indicated positive controls for both species respectively.

[Table/Fig-2]: PCR for *Campylobacter* species identification.

DISCUSSION

In the present study, the prevalence of *Campylobacter* infections accounts for 10.9% of acute diarrhoeal diseases among under-five children. Studies from Asia show a 17.7% isolation rate from Bangladesh [19], 8% from Tehran [14], 18% from Rawalpindi [20], and several studies from India showed varying ranges from 7% to 18% [21-24]. Studies have found the prevalence of *Campylobacteriosis* (*C. jejuni/C. coli*) in India during 2003-2010 to be 5-16% of gastroenteritis cases [6,21]. *C. jejuni* and *C. coli* rapidly became the most commonly recognised cause of bacterial gastroenteritis, causing 5-14% of diarrhoea worldwide [3].

Campylobacter mostly affects children. In the present study, most children suffering from *Campylobacter*-associated diarrhoea were less than 48 months of age. The maximum age group of children from which *Campylobacter* could be isolated in the present study was four years, while the minimum was 17 months. A study in China showed the peak incidence to be between 12 and 24 months of age [25]. In another study conducted in Rawalpindi [20], the maximum and minimum age of isolation were 48 months and three months, respectively. In Bangladesh, the maximum isolation rate was noted in children between 12 and 24 months [19]. A study from Ranchi [22] showed that the maximum isolation was in children below the age of six years and below four years of age from a study in Puducherry [23], while another study from Vellore [24] showed that the maximum rate of isolation was from preschool children. In studies conducted in Bijapur, Gulbarga, and Kolkata, the isolation was found more in the under-five age group [16,21]. The present study findings are similar to these studies and complement the prevailing age distribution pattern, which could be due to the combined effects of declining levels of maternally acquired antibodies and the weaning of the children. This emphasises that clinicians should suspect *Campylobacter* as a cause of diarrhoea in children under the age of five. In the present study, *C. jejuni* was detected in 4 (11.4%) male children and 2 (10%) female children [Table/Fig-3]. Gender distribution did not show any statistical significance. Similarly, no difference in sex distribution was noted in studies conducted in Puducherry [23] and Vellore [6]. On the contrary, a study done in Taiwan [26] showed a statistically significant difference in gender in the prevalence of *Campylobacter* enteritis.

Out of 55 stool samples, one (2%) yielded *Campylobacter* on culture, and 6 (10.9%) were positive by PCR. This finding indicates that culture is not as sensitive as PCR for diagnosing *Campylobacter* infection in the present settings. Similar findings were shown in a study conducted in France [27], where out of 23 *Campylobacter*s, only 16 were detected by culture, and PCR detected all. A multisite longitudinal study of enteric infections in Tanzania, Bangladesh, and Peru [28] found substantial under-detection of *Campylobacter* by selective culture compared to detection levels by both enzyme immunoassays and PCR. This indicates the limited sensitivity of culture compared to molecular methods in general. A study conducted in Kolkata found that molecular methods detected more infections with *Campylobacter* spp. than culture [21]. On the contrary, a study conducted in Puducherry states that culture was as sensitive as PCR [23].

Variables	Category	PCR Results for *Campylobacter*		p-value
		Positive	Negative	
Gender	Male	4 (11.4)	31 (88.6)	1.000
	Female	2 (10)	18 (90)	
Age	Less than 12 months	1 (4.3)	22 (95.7)	0.471
	12-24 months	2 (14.3)	12 (85.7)	
	24-60 months	3 (16.7)	15 (83.3)	
Clinical diagnosis	Acute GE with some dehydration	4 (33.3)	8 (66.7)	0.036
	Acute GE with no dehydration	1 (5.9)	16 (94.1)	
	Others	1 (3.8)	25 (96.2)	
Presenting complaints	Diarrhoea	1 (8.3)	11 (91.7)	0.404
	Fever and Diarrhoea	5 (19.2)	21 (80.8)	
	Diarrhoea with other symptoms	0	11 (100)	
Exposure to antibiotics	Fever with other symptoms	0	6 (100)	0.660
	No	1 (6.3)	15 (93.8)	
Exposure to animals	Yes (cattle)	1 (50)	1 (50)	0.208
	No	5 (9.4)	48 (90.6)	

[Table/Fig-3]: Association between different variables and PCR results. Numbers within brackets indicate row percentages for individual; Fisher's-exact test was applied, where 20% of expected values were less than 5; A significant association was considered at p <0.05

Almost all molecular methods report sensitivities and specificities greater than conventional culture and can be performed directly on stool specimens, with results available in 3-5 hours. One possible explanation for the poor performance of culture in the present study might be the relative difficulty in selectively isolating the organism after 48-72 hours of incubation with overgrowth of commensal bacteria. Providing optimum microaerobic incubation conditions with precise modern technologies like Anoxomat® or even using Gas Pak jar systems may be a better option; nevertheless, it is not cost-effective. Another explanation could be using only one selective medium in the present study.

The total number of pathogens identified in the present study was 12, of which six (50%) were *C. jejuni*. The distribution of diarrheagenic pathogens in the present study is similar to Allos BM, where *Campylobacter* diarrhoea was 2-7 times higher than diarrhoea caused by other enteric bacteria [3].

Of the total *Campylobacter* species isolated in the present study, all were *C. jejuni*. Similar findings were reported in a study conducted in Karnataka [16] and Puducherry [23], where all isolates were identified as *C. jejuni*. Similar findings were seen in a study conducted in Chandigarh; out of 30 *Campylobacter* isolates, 27 were *C. jejuni*, and three were *C. coli* [29]. In the present study, *C. jejuni* infection was higher in children who presented with fever and diarrhoea (19.2%). Similar findings were shown in a study conducted in Taiwan [26], where watery diarrhoea and fever are the most characteristic manifestations of illness. In a study done in Chandigarh, 83.75% presented with diarrhoea, 40.96% with fever, and 35.45% with abdominal pain [29]. In a study conducted in Karnataka, *C. jejuni* caused watery diarrhoea with abdominal pain in 55% of cases and watery diarrhoea with fever in 36% of cases [16].

The association of *Campylobacter* infection and animal exposure was not statistically significant in the present study. Similarly, a study conducted in Washington showed no increase in the risk of *Campylobacter* enteritis with animal exposure [30].

Utilising PCR enhances diagnostic precision, aiding in targeted and effective treatment for *Campylobacter* infections among children in developing countries. Early identification allows prompt treatment with appropriate antibiotics, reducing the severity and preventing complications. Understanding the prevalence of *Campylobacter* infections contributes to public health strategies, allowing for targeted preventive measures and interventions.

Limitation(s)

Due to logistic constraints, the study could not include antibiotic susceptibility testing and the detection of drug resistance in *Campylobacter* species, and screening for viruses was not done.

CONCLUSION(S)

It is crucial to include the diagnosis of *Campylobacter* infection in routine examinations for children with acute diarrhoea, as these infections are more prevalent than commonly perceived. Culture is insufficiently sensitive for diagnosing *Campylobacter* infection compared with nucleic acid-based diagnostics. Therefore, PCR is recommended, if feasible, as the preferred diagnostic modality for detecting *Campylobacter* infection in children with acute diarrhoea in developing countries. Precise diagnosis guides judicious antibiotic use and mitigates the risk of antibiotic resistance.

Declaration: The present study was previously presented as a meeting abstract at the International Meeting on Emerging Diseases and Surveillance (IMED), held in Vienna, Austria on November 10, 2018. https://doi. org/10.1016/j.ijid.2018.11.220.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Nov 20, 2023
- Manual Googling: Jan 05, 2024
- iThenticate Software: Feb 14, 2024 (9%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Nov 17, 2023**
Date of Peer Review: **Dec 21, 2023**
Date of Acceptance: **Feb 16, 2024**
Date of Publishing: **Apr 01, 2024**